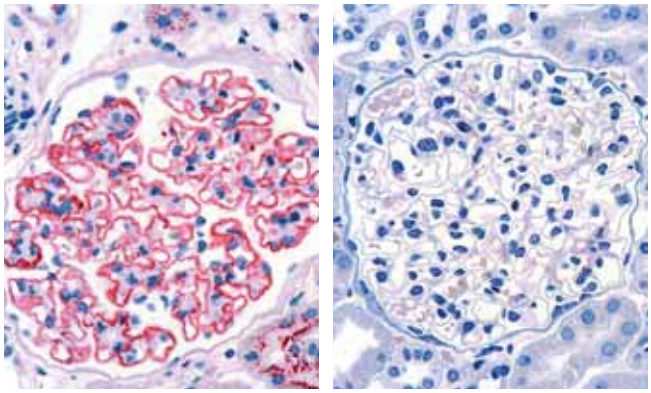
EUROIMMUN Medizinische Labordiagnostika AG

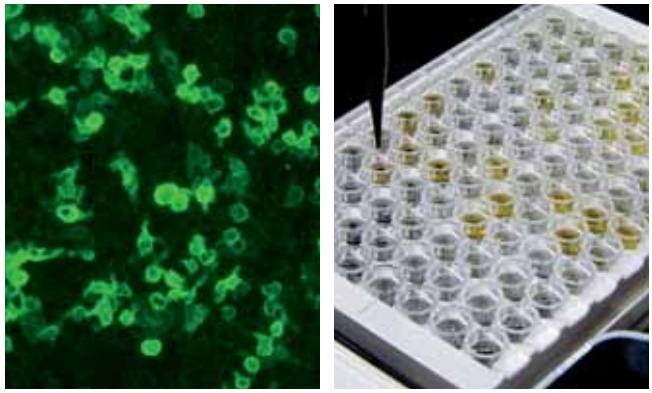
Autoantibodies against phospholipase A_2 receptors

The serological marker for primary membranous glomerulonephritis



Immunohistochemistry (IgG1 staining) of renal tissue samples

Left: inflamed renal glomeruli of a patient with pMGN; antibodies in the region of the glomerular basement membrane (red)
Right: healthy renal tissue; no antibody detection



Serological detection of autoantibodies against PLA_2R

Indirect immunofluorescence (PLA_2R-transfected cells, anti-PLA_2R positive) and microtiter ELISA

Diagnostic relevance of antibodies against PLA_2R

Primary membranous glomerulonephritis (pMGN) is associated with autoantibodies against phospholipase A_2 receptors. These are expressed in human glomeruli on the surface of podocytes. It is presumed that in vivo the antibodies trigger an inflammatory reaction after binding to their target antigen. This causes damage to the podocytes and lesions in the basement membrane. The barrier function is so strongly impaired that protein enters the urine (proteinuria).

Due to their high sensitivity and specificity for pMGN, circulating autoantibodies (IgG) against PLA_2R are an ideal diagnostic marker. They occur in 70-80 % of patients with pMGN, but not in patients with other glomerulonephritides such as lupus nephritis or IgA nephritis. The anti-PLA_2R titer has a high predictive value with respect to:

- **Disease activity and course**
  Anti-PLA_2R are highly specific for active MGN. High antibody titers are associated with a severe disease course.

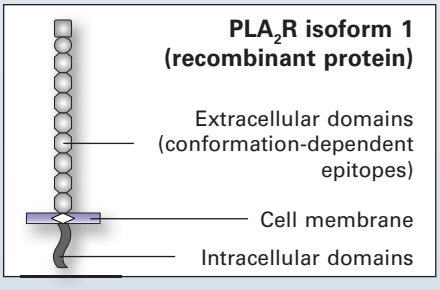
- **Therapy monitoring**
  Following immunosuppressive therapy, a decrease in the anti-PLA_2R titer is observed before an improvement in the proteinuria. An increase in the antibody titer precedes a relapse.

- **Risk assessment**
  The anti-PLA_2R titer can be used to assess the requirement for immunosuppressive therapy. Further, the risk of relapse is particularly high when high antibody titers occur after kidney transplantation.

The serological detection of autoantibodies against PLA_2R is not only easier than conventional histological investigation of renal tissue, it is less stressful for patients. Instead of kidney puncture, only a blood sample needs to be withdrawn.

How are anti-PLA_2R detected in patient blood?

Scientists at EUROIMMUN have developed state-of-the-art test systems based on **recombinant PLA_2 receptors** for the detection of autoantibodies against PLA_2R. The recombinant proteins are based on human cDNA and are produced in a human cell line. The **Anti-PLA_2R IIFT** utilises transfected fixed cells expressing PLA_2R on the cell surface as the standard substrate. For the **Anti-PLA_2R ELISA** the recombinant receptor is biochemically purified from the transfected cells and used as the ELISA antigen.



Immunhistochemical photos from Hoxha E, Kneißler U, Stege G et al., Kidney Int. 82(7): 797-804 (2012)

Anti-Phospholipase A_2 Receptor IIFT (IgG)

Order no. FA 1254-1005-50 G

The Anti-Phospholipase A_2 Receptor IIFT is an established test for serodiagnostic screening, providing qualitative and semi-quantitative analysis of human autoantibodies (IgG) against PLA_2 receptors.

In a retrospective study with 100 sera from patients with biopsy-proven primary MGN, 17 sera from patients with secondary MGN and 90 sera from patients with non-membranous glomerulonephritis, a prevalence of 52% was determined with the EUROIMMUN Anti-Phospholipase A_2 Receptor IIFT (IgG). In a control panel the specificity was 100%.

Panel (n=360)	n	Anti-PLA_2R positive
Biopsy proven primary MGN	100	52
Secondary MGN	17	0
Non-membranous GN	90	0
Healthy blood donors	153	0

Hoxha E et al., Nephrol Dial Transplant 26(8):2526-32 (2011)

Most of the studies on anti-PLA_2R performed up until now are retrospective. They include patients who might not have exhibited antibodies any more due to spontaneous remission or immunosuppressive therapy. In some of these patients the kidney biopsy and the serum sample were taken at different time points. In these cases it is possible that the serum antibodies were no longer detectable or not yet detectable again following treatment. Further, it is conceivable that patients with biopsy-proven MGN and a negative result for anti-PLA_2R did not have primary MGN. In paraneoplastic syndromes, for example, the trigger of disease, mostly a tumour, is often only detected up to two years after appearance of the kidney disorder. Such cases would be classified as secondary MGN.

Anti-PLA_2R ELISA (IgG)

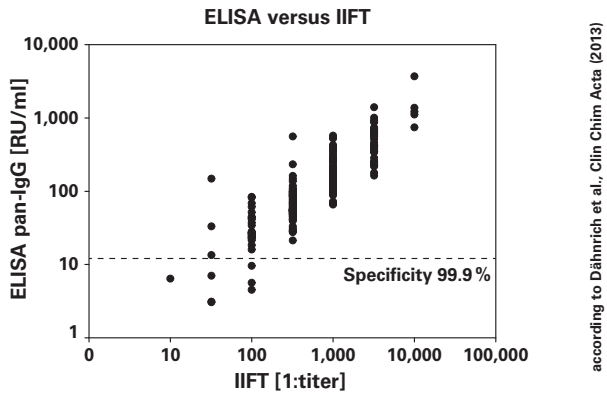
Order no. EA 1254-9601 G

The Anti-PLA_2R ELISA provides reliable determination and also quantification of autoantibodies (IgG) against PLA_2R, allowing the disease status and therapy responses of patients to be monitored.

In a study with 198 sera from patients with pMGN, 545 sera from a control panel of patients with other diseases (e.g. lupus type V, ANCA-associated vasculitis, systemic lupus erythematosis, systemic sclerosis, Sjögren's syndrome) and 291 sera from healthy blood donors, the EUROIMMUN Anti-PLA_2R ELISA (IgG) yielded a sensitivity of 96% with respect to the IIFT. The specificity amounted to 99.9% with borderline sera included.

Panel (n=1061)	n	Anti-PLA_2R positive/borderline
Primary MGN[1,2,3]	198*	190
Sensitivity	**198**	**96.0%**
Other glomerulonephritides[1,2]	230	0
Other autoimmune diseases	315	0
Healthy blood donors	291	1
Specificity	**836**	**99.9%**
Secondary MGN[1,2,3]	27	0

*Sera from pMGN patients with positive IIFT result for anti-PLA_2R antibodies
[1]R. Stahl, University Clinic of Hamburg-Eppendorf, Hamburg, Germany.
[2]J. Wetzels, University of Radboud, Nijmegen, Netherlands.
[3]G. Lambeau, CNRS, University of Nizza-Sophia Antipolis, Valbonne, France.



The results from the two EUROIMMUN test systems were in good agreement. Samples that were negative in the Anti-PLA_2R ELISA generally gave low titers of 1:10 to 1:100 in the IIFT. All sera with antibody titers of over 1:100 in the IIFT were also positive in the ELISA.

Literature

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EUROIMMUN AG · D-23560 Luebeck (Germany) · Seekamp 31 · Tel +49 451 58550 · Fax 5855591 · E-mail euroimmun@euroimmun.de
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